Cardiac Muscle Regulation II

2999-Pos Board B429
Cardiac Over-Expression of Creatine Kinase Improves Function in Failing Myocytes
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Aims: Abnormal energy metabolism contributes to heart failure (HF) and the failing heart is energy starved. Here we tested whether augmented CK energy metabolism improves myocyte dysfunction in experimental HF.
Methods and Results: We tested the response to the β-agonist isoproterenol (2.5 nM, ISO) in cardiomyocytes isolated from wild-type (WT) mice and mice over-expressing cardiac myofibrillar and mitochondrial CK (CK-M and CK-mito) from sham and HF (8 wk transverse aortic constriction, TAC) hearts, to dissect whether over-expressing CK-M or CK-mito might alter myocyte function at baseline or after an increase in energetic demand. At baseline, there were no differences in sarcomere fractional shortening (FS) or whole Ca^{2+} transient amplitude in response to ISO among sham WT, CK-M or CK-mito myocytes. However, ISO impact on FS, Ca^{2+} transient, time to 50 Ca^{2+} decay, and sarcomere re-lengthening were all reduced in WT TAC hearts, consistent with prior reports. Conversely, over-expressing CK-M or CK-mito rescued ISO-induced inotropy in TAC myocytes. No sizable differences in ISO response were noticed in cells obtained from sham WT, CK-M or CK-mito hearts. To test whether over-expressing CK-M or CK-mito confers a degree of protection against acute oxidative stress, non-TAC myocytes were exposed to H_2O_2 (50 μM for 10 min). The interval between the beginning of H_2O_2 superfusion and the appearance of an irreversible arrhythmia was measured. WT and CK-M myocytes showed a similar response (359 ± 87s *vs.* 370 ± 60s, n=5), whereas in CK-mito this interval was prolonged (580 ± 74s).
Conclusions: Over-expressing CK-M and CK-mito under failing-TAC conditions improves myocyte contraction and relaxation, likely through preserved Ca^{2+} handling; however, only the up-regulation of CK-mito can effectively buffer ROS, especially those of mitochondrial origin.

3000-Pos Board B430
Acute Ablation of Cardiac Myosin Light Chain Kinase Decreases Cardiac Performance
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Cardiac myosin light chain kinase (cMLCK) phosphorylates a single site in the regulatory light chain (RLC) of myosin to increase Ca^{2+} sensitivity of myofilament contractions. A constellation of contractile protein phosphorylations in addition to RLC phosphorylation fine-tune actin-myosin myofibrillar force development to modulate cardiac performance. In the normal beating heart RLC is significantly phosphorylated (~45%) which may play a constitutive physiological role to enhance cardiac performance. Conventional cMLCK knockout mice have dilated hearts with severely compromised cardiac performance at 10 weeks of age and older. To determine if the dilated phenotype caused by cMLCK knockout is preceded by a loss of cardiac performance associated with decreased phosphorylation of RLC and other myofibrillar proteins, we generated an acute model for the conditional knockout of cMLCK. We optimized the minimal amount of tamoxifen necessary for cMLCK ablation and assessed cardiac performance measured as fractional shortening by echocardiography. Hearts were then harvested for analyses of protein contents and phosphorylations. Five consecutive daily i.p. injections of 0.5 mg tamoxifen per mouse were sufficient to reduce cMLCK 80 ± 2% by two weeks after the first tamoxifen injection. RLC phosphorylation was reduced to 15 ± 2% while left ventricular internal diameter at end-diastole significantly increased by 1.4 ± 0.3 mm and fractional shortening decreased 47 ± 6%. There was no evidence of compensatory hypertrophy. Troponin-I and myosin binding protein-C phosphorylation at Ser23/24 and Ser282, respectively, did not significantly change. Both phosphorylations were reduced with propranolol treatment, which had no effect on RLC phosphorylation. These results suggest that constitutive RLC phosphorylation contributes to cardiac performance in the normal beating heart.

3001-Pos Board B431
Cardiac Remodeling in the Mouse Model of Marfan Syndrome Develops Independently from Aortic and Valvular Abnormalities
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Marfan syndrome (MFS) is a multisystem disorder of connective tissue caused by mutations in fibrillin-1. Heart involvement in the Marfan syndrome patients includes aortic root dilatation, valvular insufficiency, and myocardial dysfunction; it remains unclear, however, whether alterations in myocardium are triggered by valvular and aortic pathology or they develop independently. We evaluated the age-dependent cardiac remodeling and left ventricular dysfunction in the mouse model of MFS known as Fbn1039G+/− mouse (Marfan HT mouse) using echocardiography, pressure-volume loop analyses and a number of histological and biochemical techniques.
Marfan HT mice of 2-4 month demonstrated a hypertrophic cardiac remodeling accompanied by predominant decline of diastolic function and increased TGF-β canonical (p SMAD2/3) and non-canonical (pERK ½ and pMAPK38) signaling. Hypertrophic myocardium among older HT mice (6-14 months) was associated with two distinctly different phenotypes manifesting either dilated or constricted LV chamber. Dilatation of LV chamber was accompanied by biochemical evidence of greater mechanical stress, including elevated ERK1/2 phosphorylation and brain natriuretic peptide expression in comparison with constricted heart. Diastolic dysfunction in the older HT mice was combined with significant systolic impairment. The aortic valve regurgitation was registered in 20% of constricted group and 60% of dilated, while mitral insufficiency was observed in 40% of constricted group and 100% of dilated. In Marfan HT mice, extracellular matrix abnormalities were not associated with the increase of interstitial fibrosis and non-myocyte proliferation. In the mouse model of fibrillin-1 haploinsufficiency the early onset of hypertrophic cardiac remodeling and dysfunction is not consequent to functional valvular abnormalities, but it is likely to result from deficient mechanosensing and transmission of mechanical forces.

3002-Pos Board B432
Transgenic Over-Expression of Carbonic Anhydrase III in Cardiac Muscle Demonstrates a Mechanism to Resist Acidosis
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Carbonic anhydrase III (CAIII) is an abundant protein in skeletal muscle, liver and adipose cells. A cytosolic enzyme that catalyzes conversions between CO_2 and HCO_3^- in regulating intracellular pH, its physiological function in muscle is unclear. Mice lacking CAIII showed lower than wild type intracellular pH in skeletal muscle cells during fatigue treatment. To further understand the role of CAIII in muscle functions and stress adaptation, we developed transgenic mice overexpressing CAIII in the heart under the control of a cloned myosin heavy chain promoter for phenotype comparisons with wild type mouse hearts that are CAIII negative. Three months old transgenic mice showed normal cardiac phenotypes under non-stress conditions. Cardiac function was examined using *ex vivo* working heart preparations under normal and low pH conditions to investigate CAIII function in pH regulation of cardiac muscle. With equilibration of 5% CO_2 generating pH 7.4 in normal Kreb's perfusion buffer, 10% CO_2 was used to lower pH to 7.0. Functional data showed that transgenic and wild type hearts had similar pumping functions under normal pH. Perfused with low pH buffer, heart functions of both groups were decreased. In comparison with wild type controls at low pH, CAIII transgenic mouse hearts showed higher left ventricular pressure development and systolic and diastolic velocities under both baseline conditions and increased afterload stress, indicating a better tolerance to acidosis. The results suggest that CAIII may function in compensating for intracellular pH under acidotic conditions, a tractive novel approach to develop new treatment of chronic congestive heart failure.

3003-Pos Board B433
Myocardial Interstitial Serotonin and its Major Metabolite, 5-Hydroxyindole Acetic Acid Levels Determined by Microdialysis Technique in vivo Rat Heart
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Aims: The aim of this study was to elucidate myocardial interstitial serotonin (5-HT) kinetics in the heart, including 5-HT reuptake and enzymatic degradation to 5-hydroxyindole acetic acid (5-HIAA) via monoamine oxidase (MAO).
Main methods: Using microdialysis technique in anaesthetized rats, we simultaneously monitored myocardial interstitial levels of 5-HT and its major metabolite, 5-HIAA, in the left ventricle and examined the effects of local administration of a MAO inhibitor, pargyline, or a 5-HT uptake inhibitor, fluoxetine.

Key Findings: Pargyline increased dialysate 5-HT concentration from 1.8 ± 0.3 at baseline to 3.9 ± 0.5 nM but decreased dialysate 5-HIAA concentration from 20.7 ± 1.0 at baseline to 15.8 ± 1.4 nM at 60-80 min of administration. Fluoxetine increased dialysate 5-HT concentration from 1.9 ± 0.4 at baseline to 6.5 ± 0.9 nM at 60-80 min of administration, but did not change dialysate 5-HIAA concentration. Local administration of ADP (100 mM) increased dialysate 5-HT and 5-HIAA concentrations. Pargyline did not affect ADP-induced increase in dialysate 5-HT concentration but suppressed ADP-induced increase in dialysate 5-HIAA concentration during 60 min of ADP administration. Fluoxetine increased dialysate 5-HT concentration at 40-60 min of ADP administration, but did not affect ADP-induced increase in dialysate 5-HIAA concentration.
Significance: Simultaneous monitoring of myocardial interstitial 5-HT and 5-HIAA levels provides valuable information on 5-HT kinetics including reuptake and enzymatic degradation by MAO, which play a role in the regulation of myocardial interstitial 5-HT levels at baseline and when 5-HT levels are elevated.

3004-Pos Board B434

The Treatment Benefit of Ghrelin on a Mouse Model of Inherited Dilated Cardiomyopathy Caused by Troponin Mutation

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The therapeutic effect of ghrelin has been reported in humans as well as in animal models of chronic heart failure. However, little is known about the therapeutic efficacy of ghrelin for the treatment of inherited forms of dilated cardiomyopathy (DCM). We aim to examine whether ghrelin is beneficial for the treatment of inherited DCM with a deletion mutation ΔK210 in the cardiac troponin T (cTnT) gene using a knock-in mouse model. Ghrelin (150 μg/kg/day) was administered subcutaneously to the mouse model of inherited DCM. The therapeutic effects were examined on the basis of survival and myocardial remodeling. Ghrelin administration prolonged the life span of DCM mice compared to the saline-treated controls. Echocardiography data showed that ghrelin reduced left ventricular (LV) end-diastolic dimensions and increased LV ejection fraction. Moreover, histoanatomical data revealed that ghrelin decreased the heart-to-body weight ratio, prevented cardiac remodeling and fibrosis, and markedly decreased the expression of brain natriuretic peptide. Telemetry recording and heart rate variability analysis showed that ghrelin suppressed the excessive cardiac sympathetic nerve activity (CSNA) and recovered the cardiac parasympathetic nerve activity. Ghrelin has therapeutic benefits for the treatment of DCM with ΔK210 mutation in cTnT. Importantly, these cardiovascular benefits of ghrelin are likely linked to the suppression of CSNA and recovery of cardiac parasympathetic nerve activity.

3005-Pos Board B435

The Cardiac Troponin T Mutant Missing the N-Terminal Extension Causes Dose-Dependent Effects on Cardiac Function and Remodeling in Transgenic Mice

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The N-terminal extension (NTE; residues 42-73) of mouse cardiac troponin T (TnT) desensitizes cardiac myofilaments to Ca^{2+} by stabilizing thin filaments in the blocked-state. We arrived at this conclusion using detergent-skinned muscle from transgenic (TG) mouse hearts that expressed 54% of chimeric TnT (residues 1-73 of mouse cardiac TnT were replaced by residues 1-41 of mouse fast skeletal TnT). Here, we extended our investigation to include higher dose effects of the modified TnT on cardiac myofilament function/phenotype using detergent-skinned fiber studies and echocardiography measurements in two different TG mouse lines (TG-55 and TG-64 that expressed 55% and 64% of chimeric TnT, respectively). Both TG-55 and TG-64 mice showed a similar increase in myofilament Ca^{2+} sensitivity at sarcomere lengths (SL) of 1.9 and 2.3 μm. However, Ca^{2+}-activated maximal tension increased significantly only in TG-64 mice at either SL. There was a progressive decrease in the overall heart size and heart-to-body weight ratios in both TG-55 and TG-64 mice. Left ventricular diastolic functional parameters (isovolumic relaxation time and E-wave deceleration time) showed a graded increase in TG-55 and TG-64 mice; however, such effects were only significant in TG-64 mice, suggesting impaired relaxation. Systolic functional parameters (stroke volume, ejection fraction and fractional shortening) were unaffected in TG-55 mice, but significantly decreased in TG-64 mice. Thus, higher levels of chimeric TnT (64%) depressed both diastolic and systolic function significantly in TG-64 mice. We will discuss the link between the effects of the modified N-terminus of TnT on cardiac myofilament function and the resultant pathological remodeling of the heart. Our findings have pathological relevance because a growing number of disease-related mutations are found both in and near the NTE of cardiac TnT.

3006-Pos Board B436

Functional Effects of the H1-Helix of Rat Cardiac Troponin T on Cross-bridge Detachment Rate is Differently Modulated by α- and β-Myosin Heavy Chain Isoforms

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The primary structure of the H1-helix of troponin T (TnT) varies among different types of striated muscles. Moreover, these muscles also express different myosin heavy chain (MHC) isoforms. Recently, we demonstrated that pseudo-phosphorylation of residue 204 (near the H1-helix) of cardiac TnT affected the functional state of the thin filament differently in fibers that expressed either α- or β-MHC isoforms (Michael et al., Basic Res Cardiol, 109:442, 2014). In this follow-up study, we investigated how the isoform-specific function of the H1-helix of cardiac TnT was influenced by α- and β-MHC isoforms. We generated a mutant rat cardiac TnT (RfsH1) in which the cardiac H1-helix was replaced by the fast skeletal H1-helix. Recombinant RfsH1 was reconstituted into detergent-skinned cardiac muscle fibers from either normal rats (expressing α-MHC) or propylthiouracil treated rats (expressing β-MHC). Steady-state and dynamic measurements were carried out at sarcomere length 2.3 μm. Our results demonstrated that RfsH1 decreased Ca^{2+}-activated maximal ATPase activity differently in α-MHC (~33%) and β-MHC (~17%) fibers. Furthermore, RfsH1 decreased tension cost (~31%) and crossbridge (XB) distortion dynamics (~25%) in α-MHC but not in β-MHC fibers. Because the above mentioned parameters are indices of the rate of XB detachment, our results suggest that the interplay between the RfsH1- and α-MHC-mediated effects on the thin filament modulates XB detachment kinetics. Our findings suggest that the conformational changes in the H1-helix of TnT are sensitive to MHC isoform-mediated changes in the thin filament.

3007-Pos Board B437

Engineering Cardiac Troponin C: Potential Therapeutic for Heart Failure

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We have engineered cardiac TnCs with increased (L48Q) or decreased (D73N) Ca^{2+} sensitivity. To express these proteins in the *in vivo* heart we utilized an adeno-associated virus serotype 9 (AAV-9). The Ca^{2+} desensitized D73N TnC recapitulated a dilated cardiomyopathy phenotype and depressed function as observed by echocardiography and isolated cardiomyocytes. On the other hand, AAV-9 containing the Ca^{2+} sensitized L48Q TnC did not cause any disease phenotype or arrhythmias commonly associated with increased myofilament Ca^{2+} sensitivity. In healthy mice, L48Q TnC increased myocyte contraction and whole heart contractility with improved cardiovascular performance (increased V02max). Excitingly, L48Q TnC expressing mice were able to preserve higher contractility, ejection fraction, cardiac performance and decreased death rate even after undergoing trans-aortic constriction or myocardial infarction. Additionally, L48Q TnC was able to increase contractility, ejection fraction and cardiac performance in mice which expressed L48Q TnC after having a myocardial infarction. In summary, engineered TnCs show potential to be used as treatment strategies against different cardiomyopathies.

3008-Pos Board B438

Modeling the Response of Cardiac Troponin C to Calcium on the Thin Filament: Effects of Disease-Related and Post-Translational Modifications

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Calcium binding to and dissociation from cardiac troponin C (TnC) are essential steps leading to cardiac muscle contraction/relaxation. It is well documented that the calcium binding properties of TnC are not constant, but are sensitive to complex interactions between the additional thin and even thick filament proteins. There is a growing body of evidence that protein modifications/mutations within